Some Historical Elements of Microbial Ecology

2

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Abstract

We present briefly, first, the history of the discovery of microorganisms and particularly bacteria with the pioneering works of Antoni van Leeuwenhoek, Louis Pasteur, and Robert Koch, essentially. In a second and more detailed part, the history of microbial ecology is presented with particularly the very important work of Sergei Winogradsky and his discoveries of the main bacterial groups active in biogeochemical cycles. It is followed by a description of the major microbial ecologists who have been very active in promoting and developing microbial ecology throughout the world. Their role in the advances of microbial ecology is presented and discussed.

Keywords

Antoni van Leeuwenhoek • History of microbiology • Louis Pasteur • Microbial ecology • Microorganisms discovery • Robert Koch • Sergei Winogradsky

2.1 Introduction

Ecology, from the Greek words "oikos" (the house and its operation) and "logos" (knowledge, discourse, laws), can be defined as "knowledge of the laws governing the operation of the 'house,'" i.e., the science that studies the relationships between organisms and their biotic and abiotic environments. Thus, the German biologist Ernst Haëckel (1866) was the first to propose this word and to use it in that sense. By extension, microbial ecology is the science that studies the interrelationships between microorganisms and their biotic and abiotic environments. Since the early 1960s, this term has been commonly used and this discipline has developed considerably. These studies have been mainly focused on determining the role of living organisms and especially microorganisms in maintaining the ecological equilibrium of ecosystems and the wider environment. Since the 1970s, ecology has become popular as it developed not only into a science but also turned into a major social and political issue. Later, microbial ecology became a full-fledged discipline of microbiology. Today, it is particularly necessary in view of the marked deterioration of our environment and the need to "make the house livable" for the survival of humanity and the wider living world (biodiversity), that is to say, for the maintenance of quality and ecosystem balance in our environment. This discipline requires knowledge of microorganisms, their biodiversity, and their role in their immediate surroundings but also, to know at the cellular level, their metabolism and functional abilities. Thus, in this discipline, it is necessary to analyze closely the links between field approaches that study

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J.-C. Bertrand et al. (eds.), *Environmental Microbiology: Fundamentals and Applications: Microbial Ecology*, DOI 10.1007/978-94-017-9118-2_2,

microorganisms in their environment and in pure culture, which, although microorganisms have been isolated from their environment, can permit to understand them from a functional point of view and thus their metabolic potential. It is through these global studies that scientists have been able to demonstrate the key role of microorganisms in the balance of biogeochemical cycles, in the restoration of environments polluted by various chemicals, in the biodegradation or biotransformation of persistent organic pollutants, of heavy metals, etc. Thus, microbial ecology is now becoming an important discipline whose practical implications are obvious and at the service of human activities that aim at maintaining our environment livable and make development sustainable.

2.2 On the Path to Discovery of Microorganisms

Although microorganisms are present all over the earth where they are the most abundant living beings, it was not until the mid-seventeenth century with the invention of the microscope that they were actually seen. An English philosopher, Robert Hooke, was the first to observe and to describe in 1655, using a microscope, various types of fungi (molds) and protozoa. However, it appears he was not able to observe bacteria, given the low quality of the lens he used. Soon after, a French scientist Louis Joblot (1645–1723) constructed different types of microscopes with which he observed the morphologies of many microorganisms. Born in Bar-le-Duc, he was appointed "Royal" professor of mathematics and taught mathematics and geometry at the Royal Academy of Painting and Sculpture in Paris; he devoted much of his time to develop microscopes with which he described many "animalcules" and other microscopic "snakes, fish and eels" he observed in infused preparations of various plants or vinegar, and "the largest part is invisible to the ordinary scope of our eyes." He published his descriptions and drawings in a book published in 1718: "Descriptions and uses of several new microscopes and New observations on many insects, and on Animalcules which are present in prepared liquors and those are not so" (Lechevalier 1976; Le Coustumier 2010). Among unicellular microorganisms, he observed probably bacteria ("tiny eels and snakes of vinegar").

At the same time, Antoni van Leeuwenhoek (1674–1723, Fig. 2.1a), a cloth merchant and amateur scientist from Delft (Holland), created a very simple type of microscope with higher magnification (Leeuwenhoek microscope, Fig. 2.1b) to control the quality of the threads of sheets and other woven wares for commercial purposes (Dobell 1923). By observing different samples (pond water, maceration of plants, biological fluids, etc.), he was surprised to find through his microscopic lenses tiny organisms with particular forms "apparently moving with a purpose." He thus watched many types of single-celled eukaryotes and even bacteria. In a series of letters to the Royal Society in London, he described and drew different bacterial forms (cocci, rods, spirilla), indicating their movement and behavior when they were placed under different physicochemical conditions, paving the way for the first microbial ecology observations. With his observations and discoveries of microbes in different biotopes, he firmly opposed the supporters of spontaneous generation, who at that time, believed that microorganisms resulted from the decay of organic matter and not the reverse. For example, in that perspective, meat "engendered" microorganisms through its own decay. A century later, the Italian naturalist Lazzaro Spallanzani (1729–1799), in order to disprove this theory, showed that the decomposition of organic substances was caused by microorganisms that were not spontaneous but that these multiplied by cell division and could be eliminated by heating. He tried to explain to proponents of spontaneous generation that substances heated and separated from ambient air in a tight manner could not decompose and yield microorganisms, but could be kept without microbial attacks. However, his works were not totally conclusive because some samples contained bacteria capable of withstanding the heat treatments he used (spore-forming or heat-resistant bacteria). These results have provided arguments for the theory of spontaneous generation which persisted in spite of that work. Charles Cagniard-Latour (1838), Theodor Schwann (1837), and Friedrich Kützing (1837) have, in turn, attempted to disprove this theory by highlighting the role of yeasts in the fermentation of sugars to alcohol: fermentation was shown not to occur in the absence of yeast. They described the yeasts present in fermenting wine and beer and showed their role in the fermentation of sugars. In addition, Schwann (1837) demonstrated the role of airborne microorganisms in the breakdown of sugars by an experimental setup (vials with a narrow tube heated to incandescence), which allowed to leave sugars in contact with air while preventing the penetration of microbes in the heated and sterilized tubing. In a control flask open to air, organic matter was decomposed while in the bottle with the heat-sterilized narrow tube, organic matter in contact with sterilized air did not decompose. However, these experiences were not enough to convince supporters of the spontaneous generation theory who persisted in maintaining that heating of the tubes to incandescence modified the air quality in the bottles and that "disturbed air prevented the phenomenon of decomposition and spontaneous generation." It was not until the work of Louis Pasteur, who confirmed in a series of experiments (Pasteur 1857, 1860) the role of microorganisms in fermentation, that the theory of spontaneous generation was definitely disproved. Using narrow tubing vials, curved (swan

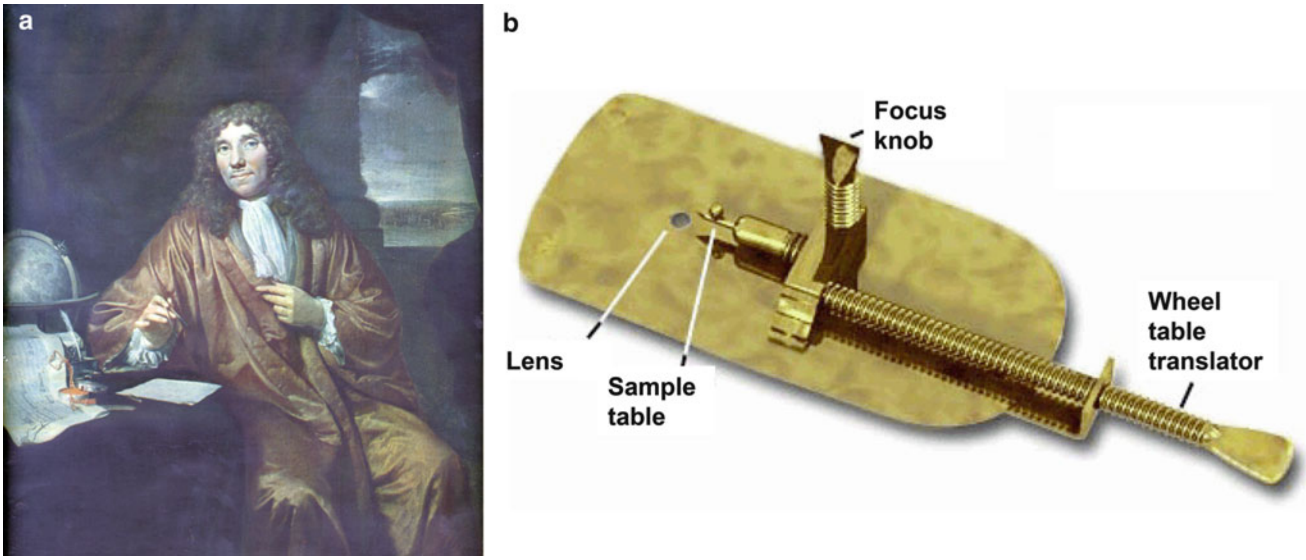

Fig. 2.1 (**a**) Antoni van Leeuwenhoek (1632–1723) (Copyright: courtesy of the Archives of the School of Microbiology of Delft, Technological University of Delft). (**b**) Microscope of A. van Leeuwenhoek (magnification × 200) (Modified from Wikipedia)

neck flasks, Fig. 2.2a) and unheated so as "not to disturb the air," he brought to a boil organic fluids where he could show, through microscopic observations and methods of bacterial cultures, that sterilized liquids were not recontaminated. These sealed and still sterile vials can still be seen in the Pasteur Museum at the Pasteur Institute in Paris. In parallel, the British physicist John Tyndall showed that air free of "particles" and optically clear did not cause degradation of organic substances, and resolved the problem of heat resistance of bacterial endospores by discontinuous heat treatments (Tyndall 1877). He was able to eliminate endospores that are resistant to boiling by successive heating cycles. The time between two heating cycles (24 h) allowed the germination of spores that had resisted the first heat treatment; such germinated bacteria were then killed by a second heating to a boil. This sterilization technique has been called **tyndallization***. His works were complementary to those of the German botanist Ferdinand Julius Cohn (1876) who described the endospores of *Bacillus subtilis* and their heat resistance properties. The studies of Pasteur, Tyndall, and Cohn have shown among other things that bacteria could survive in hostile environments by producing endospores and that these spores were airborne and carried by currents and could be scattered from one ecosystem to another.

A pioneer of the theory of microbial diseases was Ignaz Philipp Semmelweis (1818–1865) of Hungarian origin, a doctor at the obstetrics ward of the Vienna hospital. He had noticed that the death rate from puerperal fever in recently delivered women varied in different wards, ranging from 1 to 2 % in a service run by midwives to 30 % in another service staffed by physicians practicing dissections that were then not followed by hand washings. In 1847, he proposed to make hand washing mandatory when moving between services, which permitted to reduce quickly the death rate (Semmelweis 1861) but also caused deep resentment from upset department heads who greatly doubted the existence of *germs* as shown from the following quotation from the department head involved: "Herr Semmelweis claims that we carry on our hands little things that would be the cause of puerperal fever. What are these little things, those particles that no eye can see? This is ridiculous! The little things exist only in the imagination of Herr Semmelweis!"

2.3 The Beginnings of Microbiology

The major developments of microbiology from 1860 until the mid-twentieth century were largely influenced by the work of Louis Pasteur (Fig. 2.2b) and Robert Koch (Fig. 2.3).

Louis Pasteur was born in Dole (France) in 1822, made parts of his studies at the "Ecole Normale Supérieure" (ENS) in Paris. He was appointed professor of physics and chemistry, successively in Dijon, at the Faculty of Sciences of Strasbourg, at the Faculty of Sciences of Lille, and finally to the ENS in Paris. Strongly interested in the socioeconomic problems of his time, Pasteur, who had been trained as an organic chemist, has very quickly begun work on fermentation because of the problems encountered by producers of wine and beer and distillers and manufacturers of vinegar. His studies on fermentation between 1857 and 1876 have

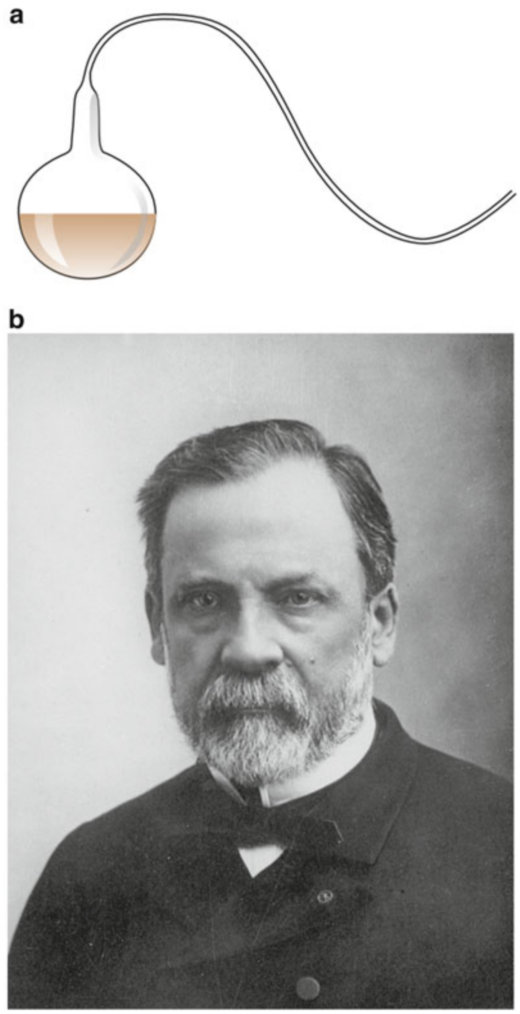

Fig. 2.2 (**a**) Gooseneck bottle used by Pasteur to study fermentation and to disprove the theory of "spontaneous generation" (see text) (Source: Wikipedia, Pasteur Institute, http://commons.wikimedia.org/wiki/file:col_de_cygne.png; free of rights). (**b**) Louis Pasteur (1822–1895) (Copyright: Institut Pasteur, Paris)

clearly established the role of anaerobic microorganisms in various fermentation processes, "fermentation is life without air" (Louis Pasteur). Indeed, during his work on fermentation, Pasteur discovered the ability of certain organisms to live in the absence of free oxygen (that is to say, in the absence of air). He called these anaerobes. (The words "aerobic" and "anaerobic" were coined by him.) From 1876 onward, Pasteur worked on different bacterial sterilization techniques: on porcelain porous filters and on the autoclave developed by Charles Chamberland (1851–1908) and the sterilization by high-temperature dry heating of the glass equipment used in microbiology (bottles, pipettes, etc.) in ovens that now carry his name (Pasteur oven).

Thereafter, he devoted much of his life to the role of pathogenic microorganisms in various infectious processes and in the genesis of infectious diseases. He thus took part in the progress of the germ theory of infectious diseases, mainly developed by Robert Koch. In 1880, Pasteur discovered the staphylococcus, which he identified as responsible for boils and osteomyelitis. Thus, for 6 years, Pasteur and Koch, scientific competitors and rivals, independently but in an intense emulation fanned by the Franco-Prussian war (1870–1871) studied infectious diseases and their causal microbes.

The last part of Pasteur's career was devoted to vaccines, that is to say, methods to prevent and suppress infectious diseases. This technique had been developed by the English physician Edward Jenner (1749–1823) who had noticed that farmworkers exposed to vaccinia, a cow disease caused by a virus similar to smallpox, which is also the origin of the name "vaccine," were subsequently protected against smallpox. Jenner had devised a standardized protocol whereby he took vaccinia blister exudates from infected workers, suspended it in water and inoculated it to children, and obtained a high rate of protection against smallpox. In the summer of 1879, Pasteur and his collaborators, Emile Roux and Emile Duclaux, discovered that chickens inoculated with old cultures of the fowl cholera microbe not only did not die but were resistant to new infections; it was the discovery of a new type of "vaccine." Contrary to what had been the case in vaccination against smallpox, it did not use as a vaccine a benign "virus"[1] (vaccinia) supplied by nature as a mild disease that immunizes against a severe disease, but the artificial attenuation of a highly virulent strain is provoked on purpose, and it is the resulting attenuated strain that is used as a vaccine. The theory of vaccination by attenuated "viruses" was confirmed by Pasteur's work on the "anthrax bacillus." After several successful results with various pathogens, prevention of rabies was the last great work of Pasteur and the only vaccination that he applied to humans. Pasteur having published his first success, his rabies vaccine soon became famous. He died in 1895 and became a national and world celebrity with streets and institutes named after him all over the world.

Robert Koch (Fig. 2.3) was born in Clausthal-Zellerfeld in Germany in 1843 and studied medicine, botany, physics, and mathematics at the University of Göttingen in 1862–1866. He was a doctor in Wollstein (Silesia) in 1876 when he published a memoir of microbiology, where he showed the role of the "anthrax bacillus" in the infectious disease. He isolated the bacterium in pure culture for the first time, demonstrated its ability to produce spores, and demonstrated its ability to induce the infectious disease. Therefore, in parallel to Pasteur, he founded in Germany

[1] At the time of Pasteur, the name "virus" was given to all infectious agents, parasites, bacteria, or real viruses; the word virus derives from Latin and means poison.

Fig. 2.3 Robert Koch (1843–1910) (Copyright: Institut Pasteur, Paris)

the discipline of microbiology. He did set up a makeshift laboratory, which allowed him to study infectious germs. Due to his discoveries, he was recruited by the Institute of Hygiene in Berlin. Koch has mainly developed general bacteriological techniques (isolation of pure strains, cultures, etc.) and has actively pursued the identification of pathogens. He isolated and identified a number of pathogens including the killer of the time that came to bear his name, Koch's bacillus (*Mycobacterium tuberculosis*), the agent of tuberculosis, and the "*Komma-Bacillus*" or *Vibrio cholerae*, the cholera agent. The announcement of pure culture of the slow-growing (15 days) tubercle bacillus was hailed at the time as a major achievement heralding the possibility of a cure. His procedure to identify the causative agent of a given infectious disease, called Koch's postulates, has four stages:

1. The suspected agent is present in sick hosts.
2. The agent must be cultivated in pure culture.
3. A pure culture can infect a healthy host, and the inoculated microorganism produces the classic symptoms of the disease.
4. The "same" microorganism can be isolated from the new hosts.

In 1887, Julius Richard Petri (1852–1921), a German bacteriologist assistant to R. Koch, invented a box that became well known in all microbiological laboratories. Robert Koch, with his assistant and his colleagues (Koch 1882, 1883), thus developed methods for the isolation and maintenance of pure cultures of many bacteria on solid media. The first tests on boiled potatoes were not very conclusive. Subsequently, Koch used gelatin in the Petri dish, and then agar, a seaweed extract, which allowed him to solidify any liquid medium regardless of its composition. This method is still used today and allows microbiologists to isolate microorganisms in pure cultures and to study them in detail. This discovery was very important because it helped to understand bacterial cells, their metabolism, behavioral changes following modifications in growth conditions, and so on. It was initially implemented mainly for the study of pathogenic bacteria but also contributed to the isolation of many environmental bacteria. However, this great discovery, which has permitted to obtain detailed knowledge of bacterial life at the cellular level through the study of pure strains, has contributed to isolate the bacteria from their environment and to study them in synthetic media that do not necessarily reflect the reality of their ecosystem. In addition, it does not permit to observe and evaluate the processes of interaction between microorganisms and their biotic or abiotic environment. Despite these remarks that highlight the limitations of this cultivation method in particular in microbial ecology, the technique proposed by Koch has deeply influenced the approaches used by most microbiologists. Actually, since the early days of microbiology and for most the twentieth century, most microbiologists influenced by Pasteur, Koch, and their students have mainly studied isolated microorganisms at the cellular or subcellular level, in order to understand their metabolic and physiological capabilities and their genetic potential. This enables today to reconstitute, at the molecular level, the metabolic and adaptive processes that govern the operation of these unicellular organisms. This knowledge is essential to understand how they function in their environment. However, it considers only microorganisms isolated and adapted to growing conditions imposed on them. Despites these limitations, his work has strongly influenced the history of microbiology, and Robert Koch was awarded the Nobel Prize for medicine and physiology in 1905. He died in 1910 in the German spa town of Baden-Baden.

The first to observe interactions between microorganisms was Sir Alexander Fleming who, in 1929, observed the inhibition of a culture of staphylococci by a contaminating mold, the fungus *Penicillium notatum* (Fleming 1929). He observed that an inhibition of the culture of staphylococci occurred, yielding a clear halo around the colony of *Penicillium*, which he hypothesized was due to diffusion of a substance secreted by the fungus. This substance, penicillin, was responsible for the great discovery of antibiotics and of the phenomenon of antibiosis in many soil bacteria that produce these molecules, giving them a competitive advantage in the fight between bacterial communities to occupy ecological niches. The field of antibiotics research has consistently expanded from thereon, for example, encompassing actinobacteria such as *Streptomyces* that the Russian-born American Selman A. Waksman showed able to synthesize a whole array of antibiotics like the aminoglycoside streptomycin that proved active against the tuberculosis agent *Mycobacterium tuberculosis*, which earned him the Nobel Prize for medicine and physiology in 1952. Microbial ecology is based on this type of observation and thus depends on the development of new methods to understand precisely such interactions. The idea of wanting to know the role and behavior of microorganisms in their biotic and abiotic environments developed from the work of Sergei Winogradsky (1856–1953, *cf.* Sect. 2.4) and has emerged from the 1970s and was instrumental in the emergence of microbial ecology. This discipline has developed alongside mainstream microbiology disciplines such as medical microbiology that studies the role of pathogenic bacteria in the genesis of infectious diseases and food and industrial microbiology that studies the metabolic role of certain bacteria for biotechnology.

Parallel to the discovery of bacteria and their role in infectious diseases, the Russian microbiologist Sergei Ivanovski (Ivanovski and Polovtsev 1890) was the first to demonstrate infectious particles of a size much smaller than that of bacteria. Ivanovski observed in the mosaic disease of tobacco leaves an infectious process caused by a pathogen unable to grow using the classical methods of microbiology and not retained on the candle filters (Chamberland candle filters made up of porous porcelain) with which one sterilized liquid by filtration and retention of bacteria on the filters. He called these particles “filterable viruses” to refer to infectious agents not retained on the filters of porous porcelain candles. He was the first to describe the tobacco mosaic virus (TMV) followed by the American virologist Rous who discovered the first animal virus in the early twentieth century (Rous 1911): the retrovirus causing leukemia in chickens. Later, the Canadian Felix d’Herelle described, in 1917, the first viruses of bacteria, called bacteriophages (d’Hérelle 1921). It was not until 1939 and the construction of the electron microscope that these viral particles could be directly observed.

2.4 The Beginnings of Microbial Ecology

The work of microbiologists mainly focused on pure cultures have partly contributed to a long-time neglect for ecological research on microorganisms. In addition, microbial ecology has had to face major methodological difficulties due to the small size of microorganisms. Thus, for a long time, mainstream macro-ecology ecologists either considered microorganisms as static particles or have generally ignored by considering their communities as “black boxes” in which their roles in biogeochemical processes are estimated by flux measurements of organic or inorganic material.

However, pioneering work in the nineteenth century has permitted to show the importance of knowledge of microorganisms in relation to their environments. In the early nineteenth century, the Swiss Nicolas-Théodore de Saussure (1767–1845) brought to light the capacity of soils to oxidize hydrogen and worked on various aspects of soil chemistry (de Saussure 1804). As this hydrogen-oxidizing capacity was inhibited by heating the soil or following the incorporation of sulfuric acid, he concluded that the oxidation activity was due to microorganisms. Similarly, the French Jacques-Théophile Schloesing and Achilles Muntz (1877) showed the oxidation of ammonium nitrate present in wastewater occurred when flowing through a sand column. The fact that this activity was destroyed by chloroform vapors and restored upon addition of a soil inoculum enabled them to conclude that it was due to the activity of microorganisms. At the same time, Pasteur was clearly establishing the role of microorganisms in the biodegradation of organic substances. He tried unsuccessfully to prove the role of microorganisms in the transformation of mineral substances, including the oxidation of ammonium nitrate, but he could not succeed. All these pioneering studies were performed on natural samples without the use of isolated bacteria.

It was not until the great discoveries of the Russian microbiologist Sergei Winogradsky (1856–1953, Fig. 2.4) from 1887 to really demonstrate the fundamental role of microorganisms in pathways of transformation of mineral compounds. He was the first to speak of the “microbiology of natural biotopes” and devoted 50 years of his life to microbiological research. He worked in several European universities.

In 1885, he left Russia and began his work in the botany laboratory of the University of Strasbourg. From 1887, he demonstrated the autotrophic ability of filamentous sulfide-oxidizing bacteria of genus *Beggiatoa*, which constituted one of his main microbial models (Winogradsky 1887). He discovered in 1988 (Winogradsky 1888) purple photosynthetic bacteria and sulfide-producing bacteria. He described taxonomically many anoxygenic phototrophic bacteria (Fig. 2.5), sulfate-reducing bacteria, and iron-oxidizing, nitrifying, denitrifying, and nitrogen-fixing bacteria. He thus discovered many metabolic pathways and showed the great diversity of microbial metabolisms. To elucidate the interactions between bacteria of the sulfur cycle, he designed an experimental device, called a “Winogradsky column” in which he was able to control the flow of nutrients and light (*cf.* Box 14.13). He could thus follow the appearance of sulfide production and development of sulfide-oxidizing purple and colorless bacteria and establish conditions for development of these microorganisms (*cf.* Sect. 14.4.3). These experiments allowed him to grow simultaneously communities of

Fig. 2.4 Sergei Winogradsky (1856–1953) (Copyright: Institut Pasteur, Paris)

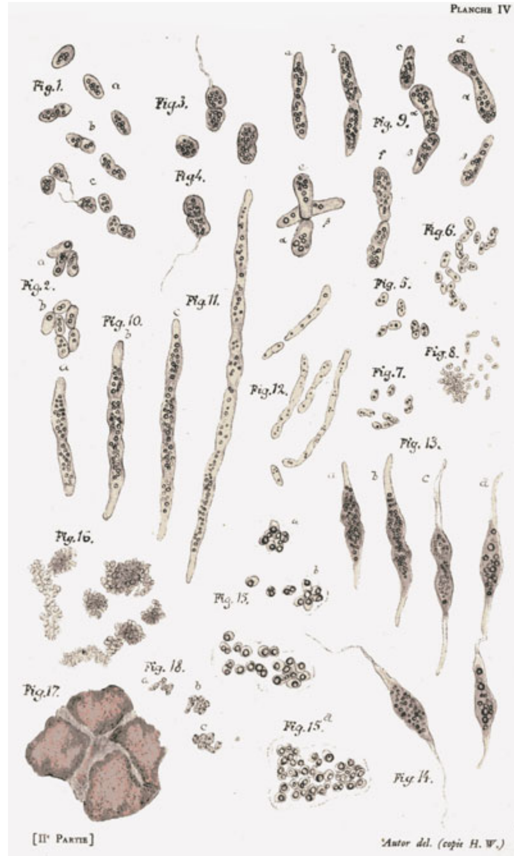

Fig. 2.5 Hand drawings by Sergei Winogradsky describing forms of phototrophic purple bacteria that appeared in his major book *Microbiologie du Sol* or (*Soil Microbiology*) published in 1949 (Winogradsky 1949, "planche IV") (Courtesy of Editions Elsevier Masson)

anaerobic, aerobic, microaerophilic, and photosynthetic bacteria along gradients of oxygen, sulfide, and light.

Then he went to Zurich in 1889 to work in a university laboratory on the problem of nitrification and proved that this process occurs in two steps, involving two groups of microorganisms. The first group performs the formation of nitrite (from the oxidation of ammonium) and the latter group the formation of nitrate (from the oxidation of nitrite to nitrate) (Winogradsky 1890).

Following these discoveries, in 1890, he was invited by Pasteur to settle in Paris but had to return to Russia where he was appointed in 1891 Head of the Department of General Microbiology at the Imperial Institute of Experimental Medicine in St. Petersburg. He remained there for 15 years, and he continued his research on nitrification, focusing on the study of the anaerobic decomposition of cellulose, used in the retting of flax. There was an especially important finding highlighting atmospheric nitrogen fixation by fermentative bacteria: he isolated an anaerobic bacillus, *Clostridium pasteurianum*, able to perform this function.

In 1906, he had to interrupt his scientific work and was appointed university professor in Belgrade in a laboratory with little means (1921).

Then he accepted the invitation of the student of Pasteur, Dr. Roux, who asked him to move to Paris to continue his research (1922). He settled in 1925 and created in Brie-Comte-Robert, close to Paris, a laboratory for the study of the natural environment, specifically soil, where he remained until 1950. He was interested especially in bacteria metabolizing nitrogen, iron, and manganese. He developed his early concepts of "ecological microbiology" and presented the summary of his work on the microbiology of natural environments, soil and water, in a book published in 1949, entitled *Soil Microbiology: Problems and Methods* which to this day is considered a classic in microbial ecology (Winogradsky 1949). Given the means of observation and analysis available to him – archaic compared to those currently available in laboratories – one can only admire the width and modernity of the microbial ecology concepts he developed. His extensive work is very difficult to summarize, but from all the results obtained from the isolation (by enrichment culture; see below Martinus Beijerinck) and the study of bacteria involved in the cycles of sulfur, nitrogen, and iron, three major concepts have emerged:

1. Chemolithotrophic prokaryotes base their metabolism on the oxidation of inorganic compounds such as ammonia

or nitrite, coupling it to the release of energy (*cf.* Sect. 3.3.2).

2. Chemolithotrophic prokaryotes are autotrophic.
3. The demonstration of the physiological process of bacterial nitrogen fixation previously discovered by Beijerinck in 1888.

Before concluding this summary of the work of S. Winogradsky, it is necessary to present his thoughts on the main methods to study microorganisms in the environment; based on the recommendations stated in his monograph, he initiated a number of research themes in contemporary microbial ecology.

Without underestimating the value of pure cultures, he insisted on the limitations of axenic cultures, "absolutely impossible in nature," that moreover remove a microorganism from its biotic and abiotic environment and place it under artificial conditions, "sometimes bordering on pathology". Another disadvantage of this approach is that the study of population dynamics is impossible. Moreover, "protected as it is in its jar," the organism is not subject to competition, and more generally it eliminates interactions with other organisms. From these remarks, he stressed the difficulty of extrapolating the results obtained in pure culture to the natural environment.

He was also aware of the diversity of the microflora of the natural environment "that provides a habitat for a swarming mass of microscopic organisms, a variety that defies imagination." He showed that few organisms can be isolated in axenic culture: the culture, on "conventional" media, thus only providing an overview of diversity. For example, he found that direct counting by microscopic examination yielded counts of billions of cells per gram of soil, while the counting of bacterial colonies on standard culture media did not exceed several tens of millions. He concluded that "therefore only a small part, comprising only 10 to 5 %, sometimes less, of the total population." It is now well established (thanks to molecular biology techniques) that only 0.1–1 % of bacteria are grown on media conventionally used in microbiology.

He also drew attention to the fact that in the natural environment, namely, the soil, "the vast majority of germs, at some point, are in a state of latent life, only a minority being in an active state." This question led to the notion of the physiological state of a microorganism that will determine its activity level and survival, in particular, its ability to grow on a culture medium, some microorganisms being "viable" but "nonculturable," unable to divide while retaining some of their physiological functions (*cf.* Sect. 15.8). About survival, especially under conditions of dietary deficiency, he felt that "the activity of the soil microbial population seems resistant to adverse conditions."

He concluded that it was necessary to use culture media that allow the development of microorganisms capable of performing a specific function and the elimination of the majority of microorganisms unable to fulfill this function; culture by enrichment is the most effective because it "reveals the diversity of functions of microbes, and allows to isolate the agents capable of fulfilling most functions including in soils: oxidation of nitrite and nitrate, oxidation of sulfur and ferrous salts, fixing gaseous nitrogen, anaerobic decomposition of cellulose and pectic substances, to name only the most interesting."

Despite reservations about classic techniques of microbiology, Winogradsky was aware that "research in Biochemistry provides us with notions of great importance in showing us the intimate ways and the chemical mechanism of life processes." Indeed, the cultivation of a microorganism fulfilling a specific function, free from competition, is essential to know its physiology (e.g., its optimum growth conditions), biochemistry (e.g., understanding the metabolic pathways of degradation of an organic compound), and genetics (e.g., presence of a gene responsible for an activity). Culture under controlled artificial conditions provides valuable clues about the ecological role of a microorganism. He also questioned the activity levels measured in the laboratory: these measures provide an estimate of the potential activity, but not the actual activity that is expressed in situ. For him, "the method of analysis of actual activities of microorganisms in nature must be based not on the behavior of isolated species outside the natural environment, but on the reactions of the entire microbial community, in this environment." It was almost impossible to achieve this at the time of Winogradsky. Technological developments presented in Chap. 17 show that serious answers are made to what remains a major concern for microbial ecologists.

Another school of soil microbiology developed in parallel in the Netherlands. Dutch microbiologist Martinus Beijerinck (1851–1931, Fig. 2.6) developed from 1905 a laboratory of microbiology at the University of Delft, the birthplace of Antoni van Leeuwenhoek. Long before the emergence of microbial ecology, he wrote that his "approach of the microbial world was within microbial ecology, that is to say the relationship between environmental conditions and forms of life that exist." He was led to the discovery of nitrogen-fixing symbiotic and nonsymbiotic bacteria (Beijerinck 1888) and was the first to isolate sulfate-reducing bacteria. He demonstrated the important processes of recycling of sulfur and nitrogen compounds in soil, highlighting the importance of biotransformations in terrestrial ecosystems and their roles in soil fertility (Beijerinck 1895). His work contributed greatly to our understanding of biogeochemical cycles and of microbial biotransformations on a global scale. Associated with the work of Winogradsky, Beijerinck's work showed the significant role of microorganisms in the recycling of elements and the balance of ecosystems necessary for the maintenance of environmental

Fig. 2.6 Martinus Beijerinck (1851–1931) (Copyright: courtesy of the Archives of the School of Microbiology of Delft, Technological University of Delft)

Fig. 2.7 Albert Jan Kluyver (1888–1956). "Rector Magnificus" of Delft University (Copyright: courtesy of the Archives of the School of Microbiology of Delft, Technological University of Delft)

quality and the maintenance of life on Earth. As a result of numerous studies, he was able to show the ubiquity of microorganisms in soils and the emergence of a community based on the selective influence of the environment. On the basis of the famous often-cited sentence "all microorganisms are everywhere, the environment selects," expressed by the Dutch microbiologist Lourens Baas Becking (see below), he developed methods to obtain and select by enrichment and isolate targeted microorganisms even if they were in low numbers in the original samples.

At the same time, other studies completed the work of Beijerinck and Winogradsky on the role of bacteria in biogeochemical cycles and confirmed the importance of emerging microbial ecology, particularly with regard to the reduction of nitrates (Deherain 1897), methanogenesis and methanotrophy (Söhngen 1906), and the isolation of hydrogenotrophic bacteria (Kaserer 1906).

2.5 Microbial Ecology During the Twentieth Century

Fundamental discoveries about the role of microorganisms in the recycling of elements in ecosystems, initiated in the early twentieth century, were pursued by a few microbiologists who continued the pioneering work of Winogradsky and Beijerinck. At the University of Delft, Albert Jan Kluyver (1888–1956, Fig. 2.7) succeeded Beijerinck as the team leader of microbiology. His team developed mainly physiological studies of soil bacteria and studied different metabolic pathways in oxidative, fermentative, and chemolithotrophic bacteria. He discovered new metabolic types and demonstrated through comparative studies, many common metabolic pathways, illustrating the diversity of the microbial world.

One of the famous students of Kluyver, Cornelius Bernardus Van Niel (1897–1985, Fig. 2.8) began his work at the University of Delft but soon joined the United States, invited by Lourens Baas Becking (1895–1963) who was a professor of physiology at Stanford University in 1928. Van Niel began a career as a microbiologist at the Hopkins Marine Station of Stanford University in California in 1929. He pursued there the tradition of the Delft School of Microbiology on comparative microbial ecophysiology. He showed in particular the existence of physiological similarities between the use of hydrogen sulfide and of water in the photosystem apparatus of plants and photosynthetic bacteria. He became interested in the ecological role, diversity, and versatility of microorganisms in soils and in different types of aquatic environments.

An excellent teacher, Van Niel was the initiator of the famous microbiology courses held there to study microorganisms in their environments. Today, annual microbial ecology courses carried out at different locations around the world continue the tradition. Through his classes, he enthused

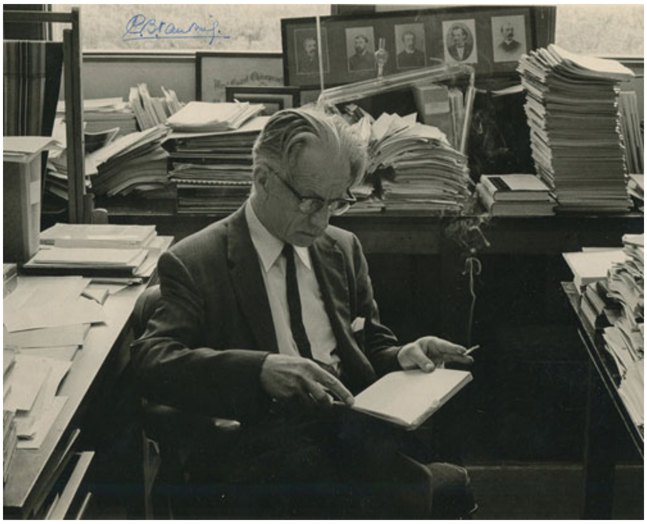

Fig. 2.8 Cornelius Bernardus Van Niel (1897–1985) (Copyright: courtesy of the Archives of the Center for History of Microbiology, American Society of Microbiology)

many students including Nobel laureates who continued his work on the physiology of bacteria, their role, and their interactions with their biotic and abiotic environments.

Another microbiologist who continued the tradition of Beijerinck and Kluyver was Hans Gunter Schlegel (1924–) in Germany. After studying under the direction of Buder in Halle, he accepted the chair of microbiology at the University of Göttingen in 1958. He contributed to knowledge on the development of purple bacteria in light gradients of lakes, as well as hydrogen-oxidizing bacteria. He was editor of the journal *Archives of Microbiology*, which has long been a journal of reference in the fields of microbiology and microbial ecology.

The work of Claude ZoBell Ephraim (1904–1989, Fig. 2.9) must also be mentioned. The scope of his investigations in the field of environmental microbiology has been very wide and has yielded over 300 publications. In the summary presentation of his results, the reader should pay particular attention to the date of publication of ZoBell's results. Indeed, he was a scientific pioneer in the study of the microbial role in corrosion from fundamental and applied points of view, the adhesion of microorganisms to solid surfaces (where he describes the different stages of development of a biofilm), and the microbiology of petroleum. On the latter topic, he particularly highlighted the role of microorganisms in the degradation of petroleum products in the environment and isolated and characterized a significant number of hydrocarbonoclastic bacteria providing extensive information on their physiology (ZoBell 1950). He also showed the ability of bacteria (especially sulfate-reducing bacteria) to synthesize certain hydrocarbons. But the most remarkable results he obtained were in marine

Fig. 2.9 Claude Ephraim ZoBell (1904–1989) (Copyright: Archives of Scripps Institution of Oceanography, University of California, San Diego)

microbiology, and he is considered the "father" of this discipline. The book he published in 1946 *Marine Microbiology* (ZoBell 1946), by the richness of the concepts presented, is a landmark document in the development of this discipline, and its reading is always recommended to researchers who wish to study the microbiology of the oceans. In this document, descriptions of the physiology and the role of microorganisms in the water column and sediment and factors controlling their distribution and activity (degradation of organic matter, nitrogen, sulfur, and phosphorus biogeochemical cycles) can be found. He brought particular attention to the effect of pressure on marine microorganisms and was the first to demonstrate the existence of bacteria capable of growing in the deep ocean. For example, during his participation to the research ship Galathea expedition, he proved with his student Richard Morita (1923–) that bacterial life was possible at depths exceeding 10,000 m and temperatures approaching 2.5 °C (ZoBell 1952; Morita 1975).

In the field of microbiology of deep-sea ecosystems, an important step was then passed with the work of Holder Windekilde Jannasch (1927–1998) and colleagues. This author who took part in numerous oceanographic cruises (about 30 in the Pacific and Atlantic Oceans, the Mediterranean and Black Sea) did develop a device for the harvesting of samples in the conditions prevailing in situ (Jannasch et al. 1973). This technological breakthrough enabled

H. W. Jannasch to isolate bacteria from their deep-sea environments by avoiding the decompression shock (Jannasch et al. 1982). Moreover, through the construction of a continuous culture system under pressure, he was able to study the kinetics of growth of barophilic bacteria at temperatures between 3 and 8 °C in the presence of low concentrations of carbon sources, both of which being characteristic of deep environments; he demonstrated that barophily, psychrophily, and oligotrophy were closely related (Jannasch et al. 1996). Through its expertise in the field of microbiology of deep environments (Jannasch and Taylor 1984), he also approached the study of prokaryotes living near the sites of deep oceanic hydrothermal vents. He thus contributed significantly to the microbiological study of these ecosystems based on chemosynthesis (*cf.* Sect. 10.3.1), in particular the role of sulfur-reducing bacteria living in symbiosis with *Riftia pachyptila*, the giant worm, devoid of mouth, digestive tract, and anus that harbors in its trophosome (*cf.* Sect. 10.3.1) endosymbiotic chemolithoautotrophic bacteria (Cananaugh et al. 1981).

With H. Jannasch many microbiologists were inspired by the teachings of Cornelius Van Niel. Among them:

– Roger Stanier (1916–1982) did his graduate studies at the University of Vancouver (Canada) and then at Stanford University (USA). In 1938, he met Prof. Van Niel at the Hopkins Marine Station of Stanford University in California during a summer course of microbiology; he then worked on the taxonomy of bacteria in Van Niel's laboratory (Pacific Grove). In 1947, he became professor of microbiology at the University of Berkeley (California, USA) and participated in the development of the *Bergey*'*s Manual of Systematic Bacteriology*, which is the reference work for identification of bacterial species. In 1957, he published with his colleagues the first edition of *The Microbial World* that has been reedited five times (Stanier et al. 1976). He contributed greatly to knowledge on photosynthetic bacteria, including the positioning of Cyanophyceae (blue-green algae) in the "kingdom of prokaryotes" and thus renamed Cyanobacteria. In 1971, he accepted the position of department head of the microbial physiology unit offered him by the Pasteur Institute; he was appointed professor at the Pasteur Institute and developed the reference cyanobacteria laboratory. He initiated with Norbert Pfennig and Moshe Shilo a series of triennial symposia on photosynthetic prokaryotes that continues to the present days, with the 14th edition in 2012.
– Norbert Pfennig (1925–2008) studied microbiology at the University of Göttingen. After his doctorate in 1957, he was appointed assistant at the same university. Stimulated by Schlegel, he devoted himself to the study of purple and green phototrophic bacteria. He then went on to work in the laboratory of Cornelius Van Niel at the Hopkins Marine Station (California, United States) where he met Roger Stanier. He then returned to the University of Göttingen as professor and, with his collaborators, developed the physiological, systematic, and ecological study of phototrophic bacteria involved in the sulfur cycle. His work brought him to isolate hundreds of strains constituting many new species and genera not only of phototrophic bacteria but also of sulfate-reducing bacteria and fermentative bacteria. He became a world reference for photosynthetic (Stanier et al. 1981) and sulfate-reducing bacteria (Pfennig et al. 1981). He was responsible for new concepts on the role of these bacteria in the biogeochemical cycles of carbon and sulfur. He showed their important role in the biodegradation of organic compounds and even some xenobiotics under anoxic conditions. He helped with the committee of the *Bergey*'*s Manual*, to reorganize the whole systematics of phototrophic bacteria with his colleague, Hans G. Trüper, professor at the University of Bonn from 1972 to 2001, founder of the Institute of Microbiology and Biotechnology of Bonn and FEMS president from 2000 to 2005. In 1980, Norbert Pfennig was appointed professor of microbiology and limnology at the University of Konstanz where he was still professor emeritus at the end of his days. Throughout his teaching career, he was renowned for his qualities as a thinker and humanist with a very open mind; he stimulated many students such as Bernhard Schink, Friedrich Widdel, Heribert Cypionka, and Jörg Overmann, who became professors of microbiology, and continued his leadership in these areas of research in microbial ecology.
– Moshe Shilo (1920–1990), born in Moscow, emigrated to Israel in 1934. He studied at the University of Jerusalem. After his appointment as professor at the University Givat Ram in Jerusalem, he founded the institute for the study of fish diseases in 1949 and the Marine Station of Eilat in 1964. He was the initiator of microbial ecology in Israel and spent his life as a researcher in the study of biotic interactions between organisms or between hosts and parasites and to the study of cyanobacteria in aquatic or marine coastal lakes. Inspired by the "Delft School" after the pioneering work of Beijerinck and after working in the laboratory of Cornelius Van Niel at the Hopkins Marine Station (United States), he became interested in the biology and blooms of cyanobacteria in aquatic environments. He initiated studies of cyanobacteria in microbial mats on which he worked with colleagues in many aquatic environments such as freshwater, marine, and hypersaline biotopes. Thus, he and his collaborators could demonstrate the existence of anoxygenic photosynthesis in cyanobacteria that are probably the origin of stromatolites, the diurnal regulatory pathways of photosynthesis and sulfate reduction in the vertical gradients established in microbial mats, and the interrelationships between microorganisms in

microbial mats. His qualities of enthusiastic researcher stimulated many students and researchers who pursued his study of microbial mats including extreme conditions of salinity (Cohen et al. 1977).

– Robert E. Hungate (1906–2004) was a pioneer of anaerobic microbial ecology. After studying at the University of Washington and then Stanford, he was the first doctoral student of Cornelius Van Niel. In 1935, he joined the laboratory of zoology, University of Texas, as a teacher and devoted himself to the study of fermentative bacteria that degrade cellulose. He initiated sophisticated methods and practices (Hungate tubes; see Sect. 17.8.2) to successfully isolate oxygen-sensitive anaerobic bacteria, fermentative bacteria, and especially methanogenic bacteria. He became interested in the termite gut and rumen of cattle. He was able to demonstrate the operation of anaerobic rumen bacterial communities and isolated syntrophic bacteria in total interdependence in the rumen. He could well describe the fermentative microbial communities and rumen methanogens which thus became the first fully characterized microbial ecosystem. His works on the rumen microbial ecology have been assembled into a book (*The Rumen and Its Microbes*: Hungate 1966) which brought a significant understanding of this unique ecosystem. With many students and collaborators, he developed the concept of the complete study of an ecosystem that requires not only knowledge of bacteria and their interactions but also a quantification of their synergistic activities.
– Ralph S. Wolfe (1921–) was educated at the University of Pennsylvania (United States) until his doctorate in 1953. After following the teachings of Cornelius Van Niel, he joined the University of Illinois as a professor where he has been professor emeritus since 1991. It was at the origin of physiological and biochemical studies of methanogenic bacteria and their role in anoxic environments and fermenters.

In France, following the work of Sergei Winogradsky, a school of soil microbiology developed at the Annex Brie-Comte-Robert of the Pasteur Institute. Professors J. Pochon, H. Barjac, and P. Tardieux developed their studies on soil microbiology including bacteria involved in the nitrogen cycle. They contributed to a better understanding of soil bacteria including their isolation from soil in two books: *Traité de microbiologie du sol* (*Treaty of Soil Microbiology*) published by Dunod in 1958 (Pochon and Barjac 1958) and *Techniques d'analyses en microbiologie des sols* (*Technical Analysis in Soil Microbiology*) (Pochon and Tardieux 1962).

In parallel, a school of soil science and soil ecology developed in Strasbourg and Nancy. In these laboratories, Yvon Dommergues (director of research at CNRS) developed studies on the physiology and ecology of soil bacteria including nitrogen-fixing bacteria. With his colleague François Mangenot, professor at the Faculty of Nancy, they wrote an important book in soil microbiology: *Ecologie microbienne du sol* (*Soil Microbial Ecology*) published by Masson in 1970 (Dommergues and Mangenot 1970). Under ORSTOM sponsorship, Yvon Dommergues created and directed the microbiology laboratory soil in Dakar (Senegal) in which he resumed the study of soil microorganisms, including studying the symbiotic nitrogen fixation and isolated first *Frankia* of *Casuarina* (Diem et al. 1983). He was very involved in the practical applications of his discoveries and participated in the installation of a green barrier of several hundred kilometers in Africa, using symbiotic nitrogen fixation, to stop erosion and expansion of the desert. Soil microbiology was then strongly developed in France by René Bardin who has worked on various aspects of nitrogen cycling in particular nitrification (Degrange and Bardin 1995).

John Postgate has made a great contribution to the study of sulfate-reducing bacteria for many years. After his doctoral studies at Oxford in the 1950s, he was employed by the Department of Chemical Research DSIR in London where he studied the physiological role of sulfate-reducing bacteria in corrosion. He later became professor at the University of Sussex in England, in the Department of Microbiology, where he studied nitrogen fixation at a biochemical level (Postgate 1998) and demonstrated the role of leghemoglobin in the legume-*Rhizobium* symbiosis and established in Brighton, United Kingdom (later moved to Norwich), an institute specialized on nitrogen fixation. He was president of the English Society for Microbiology (SGM) from 1984 to 1987.

Julian Davies, former chairman of the ASM worked on the physiology of many microorganisms, especially the secondary metabolism of *Streptomyces* and demonstrated the feasibility of the metagenomic approach for the synthesis of new metabolites (Seow et al. 1997).

David Hopwood has been the driving force behind the study of antibiotic synthesizing *Streptomyces* at the John Innes Institute in Norwich. In 1985, his group described production of the first hybrid antibiotic by genetic engineering. In 1994, he was made Knight Bachelor in the Queen's Birthday Honors for services to genetics and thus called Sir. In 2002, it was his group together with the Wellcome Trust Sanger Institute in Cambridge that published the complete genome sequence of *Streptomyces coelicolor*.

In the field of microbial ecology of extreme hyper-hot environments, we should mention the pioneering work of Thomas D. Brock (1926–). After studies at the University of Ohio (United States) and a Ph.D. in botany in 1952, Thomas Brock was formed in microbiology and molecular ecology in the research department of a company producing antibiotics. In 1960, he was appointed professor of bacteriology at Indiana University in 1971 and then as a professor of bacteriology at the University of Wisconsin-Madison, where he became director of the department of bacteriology in 1979. He devoted much of his career to the ecology of microorganisms including hyperthermophiles in the hot springs of Yellowstone Park since 1965. He discovered many bacteria and archaea and hyperthermophiles including the thermophilic bacterium *Thermus aquaticus*, which he named and from which Taq polymerase was purified, an enzyme which is widely used in molecular biology protocols that use PCR. He became interested in the boundary conditions of life on the planet and with his students he initiated research on microbial life in extreme conditions. He published the famous book *Biology of Microorganisms* which has been and still remains a basic book for many microbiology students over the world. This book has been updated regularly and is now in its 13th edition; it continues to be the reference book for many microbiologists. Thomas Brock is now a professor emeritus, and one of his students who became professor of microbiology, Michael Madigan, continues these lines of research on life under extreme conditions of high and low temperatures and on the biogeochemistry of bacteria living under these extreme conditions and continues the edition of the famous book *Biology of Microorganisms* (Madigan et al. 2010).

In the 1970s, studies of microbial ecology of biogeochemical cycles were developed under the impetus of the work of Tom Fenchel, Henry Blackburn, and Bo Barker Jørgensen at the University of Aarhus in Denmark where new methods using microelectrodes were developed to study the sedimentary gradients that develop between the oxic and anoxic conditions and microbial activities that determine in biogeochemical cycles (Jørgensen and Fenchel 1974). These micromethods were subsequently used for the study of microphysical and chemical gradients that characterize the microbial mats (Jørgensen et al. 1979; Van Gemerden et al. 1989). Fenchel and Blackburn published an important book in microbial ecology, *Bacteria and Mineral Cycling*, in which they laid the foundations of the microbial physiology of biogeochemical cycles (Fenchel and Blackburn 1979).

Regarding the environmental microbiology and applied microbial ecology, the pioneering work of Richard Bartha opened the way to studies of the role of microorganisms in the biodegradation of polluting compounds and xenobiotics. Born in Hungary, where he made some of his university studies, he participated in the uprising of 1956, and then he fled to Germany where he could prepare his microbiology doctorate with Hans Schlegel. In 1964, he joined the Department of Biochemistry and Microbiology, School of Biological Sciences and the Environment of Rutgers University (United States), where he remained professor until his retirement in 1998. He devoted his time to research on the role of microbes in the detoxication of environments contaminated with pesticides, hydrocarbons, and heavy metals, either in soil or in the marine environment. With his students including Ronald Atlas, he was responsible for the discovery of the biodegradation of petroleum compounds by bacteria thus opening the way to an important field of microbial ecology on biodegradation and biotransformation of pollutants. He showed the ability of bacteria to degrade or biotransform not only hydrocarbons including polyaromatics but also organochlorinated pesticides and heavy metals. It was at the origin of bioremediations methods to rehabilitate sites contaminated with pollutants (soils, oil spills, etc.). He became aware of the weakness of the teaching of microbial ecology, and with his student Ronald Atlas, professor of biology at the University of Louisville (United States) and former president of the American Society for Microbiology, they published the first book on *Microbial Ecology* in 1981 following a report published in 1975 in the Bulletin of the American Society for Microbiology "the teaching of microbial ecology unfortunately demands a substantial contribution with a well-documented book." They thus decided to write the book *Microbial Ecology: Fundamentals and Applications* which appeared for the first time in 1981 and, together with its fourth updated edition (Atlas and Bartha 1998), remains a basic book in microbial ecology.

Robert Mah, who was a professor of environmental microbiology at the University of California (UCLA, United States) from 1970 to 1995 and is now professor emeritus, brought a great contribution to the knowledge of methanogenic bacteria and also anaerobic halophilic bacteria.

In the field of marine microbial ecology, the work of Rita Colwell (1934–) is important. After studying microbiology and oceanography, she became professor of microbiology at the University of Maryland where she studied with many students marine microbiology in general, and especially *Vibrio* pathogens, and has shown the importance of culturability fluctuations of bacteria in the marine environment. Her pioneering work on viable nonculturable bacteria has opened the way for a whole area of research on viable nonculturable bacteria (Chaiyanan et al. 2001), which led to her being appointed to head the US NSF where she promoted work on microbial ecology. She has held many responsibilities in various American institutions where she has always promoted microbial ecology and the problem of survival of bacteria, including pathogens in aquatic environments. With Richard Morita, she contributed to an important book on marine microbiology: *Effect of the Ocean Environment on Microbial Activities* (Colwell and Morita 1974).

With regard to marine microbiology and aquatic environments, the School of Microbiology of Marseille has developed following the creation of the CNRS laboratory of bacterial chemistry by Jacques Sénez (1915–1999). After working at the Pasteur Institute in the laboratory of anaerobes with Professor Prevost, Jacques Sénez returned to Marseille where he created the laboratory of bacterial chemistry and biological corrosion in 1947; in this laboratory, he began his basic research with technological applications on sulfate-reducing bacteria and hydrocarbon biodegradation and corrosion of concrete by sulfur-oxidizing bacteria. He created, at the University of Marseille, the second Chair of Microbiology in France after Paris, in 1950. He developed his research team in 1962 and created the great laboratory of bacterial chemistry which became a reference laboratory for environmental bacteria and biotechnology, which he directed until 1983. He wrote a standard reference book in microbiology, *Microbiologie Générale* (*General Microbiology*) published by DOIN (Sénez 1968). He inspired many researchers at CNRS, ORSTOM, and at the university who continued his research paths on sulfate-reducing bacteria (Le Gall and Fauque 1988) and allowed the development of several research laboratories on marine microbiology devoted to the role of bacteria in biogeochemical cycling or in the biodegradation of hydrocarbons in the marine environment. Researchers in these laboratories contributed to the first French book for marine microbiology, *Microorganismes dans les écosystemes océaniques* (*Microorganisms in Ocean Ecosystems*) (Bianchi et al. 1989).

It has long been recognized that animal organisms harbor a large number of microbes on their surface and the Russian Ilya Ilyich Mechnikov, born in 1845, was one of the first to study them. He was formed at the University of Kharkoff in natural sciences and went on to study marine fauna at Heligoland and then to various universities (Göttingen, Munich, Giessen) where he studied intracellular digestion in the flatworm and phagocytosis. In addition to his work on immunity that earned him the Nobel Prize in Physiology and Medicine in 1908, he started the study of the flora of the human intestine and developed a theory that gut microbes' metabolites caused poisoning, which could be prevented through a diet containing fermented milk, containing large amounts of lactic acid.

The French René Dubos, born in Val d'Oise near Paris, in 1901 was recruited in the National Agronomical Institute (INRA) as an agronomist in 1921. He soon moved to work with O. T. Avery at Rockefeller University in New York where he studied enzymes that break down the capsule of pneumococci and developed gramicidin, the first commercialized antibiotic. He was elected to the US National Academy of Sciences in 1941 as a consequence of this discovery but was always an advocate that biological equilibrium was a better way to fight diseases than antibiotics. He thus coined the well-known motto "think globally, act locally" to suggest that global environmental problems can be solved only by considering ecological, economic, and cultural aspects of our local surroundings. His most popular work *Mirage of Health* (1959) is a reflection on the balance between hosts and pathogens where health is never definite because disease results from the dynamic process of life.

Microbial ecology of the digestive tract, animal and human, has experienced strong development in the group of P. Raibaud in the laboratory of INRA at Jouy-en-Josas, by using several techniques such as axenic animals and antibiotherapy, allowing to know the dominant flora at different stages of life and the changes associated with certain diseases (Ducluzeau and Raibaud 1979).

2.6 Microbial Ecology Today

Since 1970, microbial ecology has developed considerably, and many research laboratories around the world are involved in this field of research. Microbial ecologists study interactions between microorganisms and their environment or between microorganisms and other biological components of ecosystems. Therefore, they require a wide knowledge in the fields of physics, chemistry, and biology. The study of microbial ecology therefore requires close links with various disciplines for studies of microorganisms and their roles in their environments, whether in geophysics, geochemistry, soil science, limnology and oceanography, climatology, general biology, botany and zoology, biochemistry and molecular biology, and statistics and biostatistics.

The methods of molecular biology, which have greatly expanded since the 1980s, have led to major advances in all biological sciences and especially in microbial ecology. They, among others, contributed greatly to the knowledge of the diversity and adaptations of microbial communities in ecosystems. They have allowed understanding microbial interactions by gene flow and response capacities of microbial communities to environmental stresses and increasingly important inputs of toxic compounds in ecosystems. Considering these different subject areas, the community of microbial ecologists is very large today, as shown by the number of scientific articles in constant increase in the flagship journals of the discipline such as *Microbial Ecology* (quarterly since 1974), *FEMS Microbiology Ecology* (quarterly since 1985), *Applied and Environmental Microbiology* (monthly since 1976), *Environmental Microbiology* (monthly since 1999), *Geomicrobiology Journal* (quarterly since 1983), *Aquatic Microbial Ecology* (monthly since 1995), and *ISME Journal* (monthly since 2007) and in numerous journals of ecology, soil science, and aquatic or ocean studies.

Given these important developments, microbial ecologists have decided to establish an international association, *ISME* (*International Society for Microbial Ecology*), created in 1977. This society aims to enable global exchanges between microbial ecologists using Internet forums, with a scientific periodical (*ISME Journal*, which is a monthly magazine with articles of microbial ecology and information on *ISME*) and especially with symposia that have taken place every third year from 1977 to 2004 and have been held every second year since 2004. Thus, the 14 symposia held since 1977 have brought together about 1,500–2,000 participants who have found opportunities for exchange, interaction, or cooperation in the field of microbial ecology at the global level.

Many national associations have as a function to encourage exchanges between members and organize meetings. This is the case of the *ASM* (*American Society for Microbiology*) founded in 1899, the *SGM* (*Society for General Microbiology*) founded in 1945, the CSM (*Canadian Society for Microbiology*) founded in 1952, the *CSM* (*Chinese Society for Microbiology*) founded in 1952, the *SFM* (*French Society of Microbiology*) founded in 1937, and the *AFEM* (*Association Francophone d'Écologie Microbienne*) founded in 2004.

References

Atlas R, Bartha R (1998) Microbial ecology, fundamental and applications, 4th edn. Addison Wesley Longman, Reading

Beijerinck MW (1888) Die Bacterien der Papilionaceen-Knöllchen. Bot Ztg 46:725–735

Beijerinck WM (1895) Über *Spirillum desulfuricans* als Ursache von Sulfat-reduktion. Zentralbl Bakteriol Abt L 1–9:104–114

Bianchi M, Marty D, Bertrand J-C, Caumette P, Gauthier M (1989) Micro-organismes dans les écosystèmes océaniques. Masson, Paris

Cagniard de La Tour C (1838) Mémoire sur la fermentation vineuse. Ann Chim Phys 68:206–222

Cananaugh CM, Gardiner SL, Jones ML, Jannasch HW, Waterbury JB (1981) Procaryotics cells in the hydrothermal vent tube worm *Riftia pachyptila* Jones: possible chemoautotrophic symbionts. Science 213:340–341

Chaiyanan S, Huq A, Maugel T, Colwell RR (2001) Viability of the nonculturable *Vibrio cholerae* O1 and O139. Syst Appl Microbiol 24:331–341

Cohen Y, Krumbein WE, Shilo M (1977) Solar Lake (Sinai). Distribution of photosynthetic microorganisms. Limnol Oceanogr 22:609–620

Cohn F (1876) Untersuchungen uber Bakterien IV. Beitrage zur Biologie der Bacillen. Beitr Biol Pflanzen 2:249–276

Colwell RR, Morita RY (1974) The effects of the ocean environment on microbial activities. University Park Press, Baltimore

d'Hérelle F (1921) Le Bactériophage: Son rôle dans l'Immunité. Masson, Paris, 227 p

de Saussure NT (1804) Recherches chimiques sur la végétation. Vve Nyon, Paris, 327 p

Degrange V, Bardin R (1995) Detection and counting of *Nitrobacter* populations in soil by PCR. Appl Environ Microbiol 61:2093–2098

Deherain PP (1897) La réduction des nitrates dans la terre arable. C R Acad Sci Paris 124:269–273

Diem H, Gauthier D, Dommergues Y (1983) An effective strain of *Frankia* from *Casuarina* sp. Can J Bot 61:2815–2821

Dobell C (1923) A protozoological bicentenary: Antony van Leeuwenhoek (1632–1723) and Louis Joblot (1645–1723). Parasitology 15:308–319

Dommergues Y, Mangenot F (1970) Écologie microbienne du sol. Masson, Paris

Ducluzeau R, Raibaud P (1979) Écologie microbienne du tube digestif. INRA and Masson, Paris

Fenchel T, Blackburn H (1979) Bacteria and mineral cycling. Academic Press, London

Fleming A (1929) On the antibacterial action of cultures of a *Penicillium*, with special reference to their use in the isolation of *B. influenzae*. Br J Exp Pathol 10:226–236

Haeckel E (1866) Generelle Morphologie. I: Allgemeine Anatomie der Organismen. II: Allgemeine Entwickelungsgeschichte der Organismen. Reimer, Berlin

Hungate R (1966) The rumen and its microbes. Academic Press, New York

Ivanovski D, Polovtsev VV (1890) Die Pockenkrankheit der Tabakspflanze. Mem Acad Sci St Petersbourg Ser 7(37):1–24

Jannasch HW, Taylor CD (1984) Deep sea microbiology. Annu Rev Microbiol 38:487–514

Jannasch HW, Wirsen CO, Winget CL (1973) A bacteriological pressure-retaining deep-sea sampler and culture vessel. Deep-Sea Res 20:661–664

Jannasch HW, Wirsen CO, Taylor CD (1982) Deep sea bacteria: isolation in the absence of decompression. Science 216:1315–1317

Jannash HW, Wirsen CO, Doherty KM (1996) A pressurized chemostat for the study of marine barophile and oligotrophic bacteria. Appl Environ Microbiol 62:1593–1596

Jørgensen BB, Fenchel T (1974) The sulfur cycle of a marine sediment model system. Mar Biol 24:189–201

Jørgensen BB, Revsbech NP, Blackburn TH, Cohen Y (1979) Diurnal cycles of oxygen and sulphide microgradients and microbial photosynthesis in a cyanobacterial mat. Appl Environ Microbiol 38:46–58

Kaserer H (1906) Die oxydation des Wasserstoffs durch Mikroorganismen. Zentralbl Bakteriol II Abt 16:681–696

Koch R (1882) Die Aetiologie der Tuberculose. Berl Klin Wochenschr 19:221–230

Koch R (1883) Ueber die neuen Untersuchungsmethoden zum Nachweis der Mikroorganismen in Boden, Luft und Wasser. Aerztliches Vereinsblatt für Dtschl 237:244–250

Kützing FT (1837) Microscopische Untersuchungen über die Hefe und Essigmutter, nebst mehreren andern dazu gehörigen vegetabilischen Gebilden. J Prakt Chem 11:385–409

Le Coustumier A (2010) Louis Joblot et ses microscopes. Bull Soc F Microbiol 25:89–100

Le Gall J, Fauque G (1988) Dissimilatory reduction of sulfur compounds. In: Zehnder AJB (ed) Biology of anaerobic microorganisms. Wiley, New York, pp 587–640

Lechevalier H (1976) Louis Joblot and his microscopes. Bacteriol Rev 40:241–258

Madigan M, Martinko JM, Stahl D, Clark DP (2010) Brock: biology of Microorganisms, 13th edn. Pearson Benjamin-Cummings, San Francisco

Morita R (1975) Psychrophilic bacteria. Bacteriol Rev 39:144–167

Pasteur L (1857) Mémoire sur la fermentation alcoolique. C R Acad Sci Paris 45:1032–1036

Pasteur L (1860) Mémoire sur la fermentation alcoolique. Ann Chim Phys 58:323–426

Pfennig N, Widdel F, Trüper HG (1981) The dissimilatory sulfate-reducing bacteria. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes. Springer, Berlin, pp 926–940

Pochon J, De Barjac H (1958) Traité de microbiologie du sol. Dunod, Paris

Pochon J, Tardieux P (1962) Techniques d'analyses en microbiologie du sol. éditions de la Tourelle, Saint Mandé
Postgate J (1998) Nitrogen fixation, 3rd edn. Cambridge University Press, Cambridge, UK
Rous P (1911) A sarcoma of the fowl transmissible by an agent separable from the tumor cells. J Exp Med 13:397–411
Schloesing J, Muntz A (1877) Sur la Nitrification par les Ferments Organisés. C R Acad Sci Paris 84:301–303
Schwann T (1837) Vorläufige Mittheilung, bettreffend Versuche über die Weingährung und Fäulniss. Ann Chim Phys 41:184–193
Semmelweis IP (1861) Die Aetiologie der Begriff und die Prophylaxis des Kindbettfiebers. Hartleben, Pest, Vienna/Leipzig
Senez J (1968) Microbiologie générale. Doin, Paris
Seow KT, Meurer G, Gerlitz M, Wendt-Pienkowski E, Hutchinson CR, Davies J (1997) A study of iterative type II polyketide synthases, using bacterial genes cloned from soil DNA: a means to access and use genes from uncultured microorganisms. J Bacteriol 179:7360–7368
Söhngen NL (1906) Über Bakterien, welche Methan als Kohlenstoffnahrung und Energiequelle gebrauchen. Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt II 15:513–517
Stanier RY, Aldelberg EA, Ingraham J-L (1976) The microbial world. Prentice Hall, Englewood Cliffs
Stanier RY, Pfennig N, Trüper HG (1981) Introduction to the phototrophic prokaryotes. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes. Springer, Berlin, pp 197–211
Tyndall J (1877) On heat as a germicide when discontinuously applied. Proc R Soc Lond 25:569
van Gemerden H, Tughan R, de Wit R, Herbert A (1989) Laminated microbial ecosystems on sheltered beaches in Scapa Flow, Orkney Islands. FEMS Microbiol Ecol 62:87–102
Winogradsky SN (1887) Über Schwefelbakterien. Bot Ztg 45:489–600
Winogradsky SN (1888) Über Eisenbakterien. Bot Ztg 46:261–270
Winogradsky SN (1890) Sur les organismes de la nitrification. C R Acad Sci Paris 60:1013–1016
Winogradsky S (1949) Microbiologie du sol. Problèmes et méthodes. Masson, Paris
ZoBell CE (1946) Marine microbiology. Chronica Botanica Compagny, Waltman
ZoBell CE (1950) Assimilation of hydrocarbons by microorganisms. In: Nord FF (ed) Advances in enzymology. Interscience Publishers, New York/London, pp 443–486
ZoBell CE (1952) Bacterial life at the bottom of the Philippine Trench. Science 115:507–508